FORMATION AND INHIBITION OF N-NITROSOTHIAZOLIDINE IN BACON

JOHN W. PENSABENE and WALTER FIDDLER

□ N-NITROSOTHIAZOLIDINE (NTHZ) has been detected and confirmed in a variety of smoked, cured meat products (Pensabene and Fiddler, 1983a). Although the formation of this N-nitroso compound has been linked to the heating-smoking step in bacon processing (Pensabene and Fiddler, 1983b), the exact formation mechanism has not been determined. We have shown that NTHZ formation in bacon occurs prior to final cooking, since levels are higher in uncooked than in fried bacon or its drippings, and that NTHZ levels are higher on the surface of bacon than in the less-exposed interior (Pensabene and Fiddler, 1983b). Gray et al. (1982) have shown that NTHZ formation can be inhibited by the addition of α-tocopherol to the cure solution prior to pumping.

Fiddler et al. (1983) reported that NTHZ is not mutagenic when synthesized directly from thiazolidine; however, a mutagen was formed from the reaction of cysteamine–formaldehyde–nitrite, without isolating thiazolidine. While thiazolidine itself has not been reported in foods, it has been obtained by a cysteamine–D-glucose–water Browning model system reaction (Sakaguchi and Shibamoto, 1978). In addition, a mechanism for the formation of NTHZ in bacon from formaldehyde and cysteine or cysteamine was proposed by Mandagere et al. (1984). Since these precursors could be present in smoked meat products, it is possible that the mutagen observed in the formaldehyde–cysteamine–nitrite model system may be formed in bacon as well. Therefore, we undertook this study to determine the factors contributing to NTHZ formation, and also to determine the processing conditions necessary to inhibit its formation in bacon.

Samples Prepared and Analyzed

Samples were prepared and analyzed as follows:

• **Reagents.** NTHZ and N-nitrosothiomorpholine (NTMOR) were synthesized from their corresponding amines and sodium nitrite under acidic conditions, and purified by fractional vacuum distillation, as described by Pensabene et al. (1972). A complete list of reagents used for determining NTHZ in raw bacon was reported by Pensabene and Fiddler (1982). All other chemicals were purchased from local suppliers and analyzed for nitrosamine contamination prior to use.

• **Bacon Processing.** Skinned, matched pork bellies were purchased from a local slaughterhouse within 24 hr postmortem and stored at −18°C until used. Prior to processing, the bellies were thawed for 1 wk in a 1°C cooler. The thawed bellies were cut into either three or six lengthwise sections and injected with a commercial cure to approximately 110% of their green weight to achieve added target levels in the finished product of 1.5% NaCl, 0.75% sugar, and 0.3% sodium tripolyphosphate. In addition, the following components were added either alone or in combination: 120 ppm of $NaNO_2$, 550 ppm of sodium ascorbate (NaAsc), 500 ppm of α-tocopherol (1:0.4 α-tocopherol:polysorbate 20), 1,000 ppm of liquid smoke. The pumped bellies were stored in polyethylene bags at 1°C for 18 hr. For the surface treatment inhibition study, α-tocopherol (20% α-tocopherol in Neobee M-5 oil) and liquid smoke were sprayed on the surface of the bellies before and after 2, 4, and 6 hr of processing. All the bellies were then processed in a smokehouse using only slab hardwood as the source of both heat and smoke. The bellies were kept in the smokehouse with no air exchange for 7 hr, where they reached an average internal temperature of 60°C at the end of processing. The raw bacon samples were ground prior to analysis.

• **Model System Processing.** To meatless emulsions consisting of 140 mL of water, 60 mL of corn oil, 4.0 g of salt, and 3.0 g of xanthum gum were added 100 ppm of the individual potential NTHZ precursors (thiazolidine, cysteamine, cysteine, cystine, methionine, formaldehyde). The emulsions were then stuffed into cellulose casings and processed in the smokehouse under the same conditions used for the bacon experiments.

• **Analysis for NTHZ in Bacon.** The complete details of the procedure for the analysis of NTHZ in raw bacon have been described by Pensabene and Fiddler (1982). All NTHZ values reported for the bacon samples have been corrected for the recovery of the NTMOR internal standard in each individual sample. "nd" denotes "none detected" or <1 ppb, the minimum level of reliable measurement based on the gas chromatography–Thermal Energy Analyzer system response.

• **Analysis for NTHZ in Meatless Emulsions.** To 10 g of emulsion in a 250-mL beaker were added 50 mL of water and 50 mL of dichloromethane (DCM). The mixture was homogenized for 3 min using a Tissumizer (Tekmar Co., Cincinnati, OH), then the emulsion was transferred to a centrifuge bottle and centrifuged for 15 min at 6,000 rpm (0-5°C). The solution was then filtered through glass wool into a 250-mL separatory funnel, the DCM separated and collected in another 250-mL funnel, and the aqueous solution re-extracted with an additional 50 mL of DCM. The combined DCM extracts were washed once with 25 mL of 5N NaOH. The samples were dried and concentrated, then the oily residue was added to an alumina column, as described by Pensabene and Fiddler (1983). All samples, whether cured meat products or meatless emulsions, were analyzed for NTHZ in duplicate.

• **Analysis for Sodium Nitrite.** Residual sodium nitrite content was determined in 10 g of raw, comminuted sample by the modified Griess-Saltzman procedure (Fiddler, 1977).

• **Statistical Analysis.** Statistical analyses were carried out according to the methods of Snedecor and Cochran (1979).

• **Safety Note:** Precaution should be exercised in the handling of nitrosamines, since they are potential carcinogens.

Results obtained

The following results were obtained:

• **NTHZ in Meatless Emulsion Model System.** The NTHZ values resulting from the addition of several potential precursors to meatless emulsions prior to smokehouse processing are shown in Table 1. No NTHZ was detected in the control samples. Only thiazolidine and cysteamine yielded significant quantities of NTHZ in the finished product. These results are similar to those previously reported (Fiddler et al.,

The authors are, respectively, Research Chemist and Research Leader, Eastern Regional Research Center, Agricultural Research Service, U.S. Dept. of Agriculture, Philadelphia, PA 19118

1982), in which these same precursors were added directly to ground, commercial bacon prior to frying. Since the slab hardwood was the only source of both heat and smoke, it appears that nitrogen oxides generated from the burning wood were the principal source of nitrosating species in this experiment. In addition, the results suggest that NTHZ was not preformed in the smoke, then deposited on the product; but rather that a smoke component, probably formaldehyde, reacted with cysteamine or cysteine in the model system to form thiazolidine or its 4-carboxylic acid derivative, as proposed by Mandagere et al. (1984). However, since processing conditions were identical to those used for the bacon, the results suggest that the principal route to NTHZ is not through the nitrosation of thiazolidine-4-carboxylic acid (THZC), since no detectable NTHZ values, upon addition of cysteine, were found. It is also interesting to note that as little as 10 ppm of either thiazolidine or cysteamine still yielded a significant amount of NTHZ in the nonmeat emulsion; this indicates that these precursors need be present in meat only in small quantities, and that the nitrosation reaction is very facile, as one would expect from a weakly basic amine (Mirvish, 1975).

Table 1—**Effect of potential precursors** *on nitrosothiazolidine formation in meatless emulsions*

	NTHZ Concentration (ppb)			
	Experiment			
Precursor[a]	1	2	3	4[b]
Control	nd[c]	nd	nd	nd
Thiazolidine	564	1,149	1,458	343
Cysteamine	69	112	574	105
Cysteine	nd	nd	nd	—
Cystine	nd	nd	nd	—
Methionine	nd	nd	nd	—
Formaldehyde	nd	nd	nd	—
Sodium nitrite	nd	nd	3	—

[a]100 ppm added precursor
[b]10 ppm added precursor
[c]None detected

● **Effect of Nitrite Level.** Previously, we reported that no correlation was evident between residual sodium nitrite and NTHZ levels in either raw bacon or other cured meat products (Pensabene and Fiddler, 1983a; b). However, to determine the effect of ingoing sodium nitrite levels on NTHZ values, eight pork bellies were cut into thirds, pumped with a nitrite cure (120 ppm, no ascorbate), a nitrite-free cure, and an unpumped control, then processed and analyzed for NTHZ. The data are shown in Table 2 and the statistical summary of the results in Table 3. There was a highly significant difference ($P < 0.01$) in NTHZ among bellies, as expected. There was also a highly significant difference in NTHZ levels among the three treatments and evidence of highly significant belly × treatment interaction. Further investigation by individual contrasts indicated a highly significant difference between the nitrite-cured bellies and the two no-nitrite treatments. No significant NTHZ difference ($P < 0.05$) was found between the control and nitrite-free treatments. These results show that the presence of nitrite in the cure, prior to smokehouse processing, significantly increases NTHZ levels in the finished product (approximately sixfold). Nitrogen oxides from the smoke can also serve as the nitrosating agent to cause NTHZ formation in bacon, but they appear to have a minor role compared to the nitrite contained in the product.

● **Effect of Inhibitors.** The effect of potential inhibitors on NTHZ formation in bacon was also investigated. The NTHZ data are shown in Table 4 and the statistical analysis in Table 5. As expected, there was a highly significant difference ($P < 0.01$) among bellies and among treatments, and a highly significant interaction between treatments and bellies. Further statistical analysis by simple contrasts showed no significant difference in the NTHZ values among the cure-incorporated ascorbate, ascorbate combined with α-tocopherol, and sprayed liquid smoke treatments. Also, there was no significant difference among the control, cure-incorporated α-tocopherol, and sprayed α-tocopherol treatments. However, in this latter group, NTHZ was significantly higher than in the former group. We also found that neither α-tocopherol treatment (cure addition or spray) reduced the NTHZ content in the raw bacon. This finding was contrary to that reported by Gray et al. (1982), who observed inhibition of NTHZ in fried bacon when 500 ppm of α-tocopherol was added to the cure prior to pumping. This difference in results may be due to differences in the mode of α-tocopherol addition (cure-solubilized in polysorbate 20 vs salt-coated tocopherol plus lecithin), the substrate analyzed (raw vs fried bacon), or the method of analysis (dual-column chromatographic vs mineral-oil distillation method). The mineral-oil distillation method has been shown to artifactually produce nitrosamines (Pensabene and Fiddler, 1982), except when an antioxidant nitrosamine inhibitor or a nitrite-destroying compound is added prior to distillation (Hotchkiss et al., 1980).

Our finding that ascorbate is more effective than α-tocopherol in reducing NTHZ formation in smoked, raw bacon is also contrary to the results we previously obtained with bacon cured with these two compounds, then analyzed, after frying, for N-nitrosopyrrolidine, NPYR (Fiddler et al., 1978). In those experiments, α-tocopherol inhibited NPYR formation, but it had little effect on residual nitrite prior to frying. The effectiveness of α-tocopherol was attributed to the fact that the NPYR precursors were in the bacon adipose tissue rather than in the lean tissue (Fiddler et al., 1974), and the fact that α-tocopherol is lipid-soluble. Thus, in the case of NTHZ, reduction by sodium ascorbate could be due to the hydrophilicity of NTHZ precursors or, more likely, to the reduction of residual nitrite levels, thereby making less nitrite available for NTHZ formation. This hypothesis was supported by statistical analysis of the residual sodium nitrite data, which showed that the α-tocopherol treatments (either in the cure or sprayed on the surface) did not significantly ($P < 0.05$) reduce the residual nitrite levels compared to the control; whereas the sodium ascorbate treatments (either alone or in combination with α-tocopherol) did. Therefore, NPYR inhibition, previously observed for α-tocopherol, must occur at the time of frying. Since raw, not fried, bacon was analyzed in this study, the effect of α-tocopherol inhibition on NTHZ was not observed. However, the inhibitory mechanism of ascorbate is clearly different, since it takes place in the raw bacon, and is consistent with the observed reduction in residual sodium nitrite.

● **Effect of Liquid Smoke.** Trace quantities of NTHZ were previously detected by us in some commercial liquid smoke solutions, and were considered to contribute to a minor extent to the amount of NTHZ found in bacon (Pensabene and Fiddler, 1983a). The results of our present study show that liquid smoke sprayed on the surface of cured pork bellies prior to and during processing can also significantly inhibit NTHZ formation. This inhibition may be due to the acidic nature of the liquid smoke solutions, which could both destroy nitrite in the product, as evidenced by the low residual nitrite values in Table 4, and cause NTHZ to decompose. The acidity of the smoke ($pH < 3$) and the low pH (4.4-4.9) of the product resulting from the use of a lactic acid starter culture may help explain why the NTHZ content of commercial Lebanon bologna was low (Pensabene and Fiddler, 1983a), considering that it is subjected to a prolonged smoking cycle (in excess of 4 days). In addition, the phenolic compounds in the liquid smoke may compete with the NTHZ precursor(s) for the nitrosating species to form C-nitro- and C-nitrosophenols. There is some evidence for the formation of these latter compounds, since several have been identified in liquid smoke solution–nitrite model systems (Knowles et al., 1975) and in smoked bacon (Knowles et al., 1974, Gilbert et al., 1975). The net result of the nitrosation of smoke phenols may be reduced concentrations of NTHZ. However, the role of phenols in nitrosamine formation is not clearcut, since p-nitrosophenol has been reported to have a catalytic effect on the nitrosation of diethylamine (Walker et al.,

Table 2—**Effect of added sodium nitrite** *on NTHZ formation in bacon*

	Concentration[a]					
	Control (no cure)		Nitrite-free cure		Nitrite cure	
Experiment No.	$NaNO_2$ (ppm)	NTHZ (ppb)	$NaNO_2$ (ppm)	NTHZ (ppb)	$NaNO_2$ (ppm)	NTHZ (ppb)
1	1	nd	7	2.38	70	7.13
2	1	2.05	3	2.24	56	18.32
3	1	1.37	2	1.73	71	15.43
4	nd	1.11	2	nd	50	5.09
5	nd	nd	1	nd	46	8.46
6	nd	nd	nd	nd	39	3.68
7	nd	2.46	nd	1.06	42	5.29
8	nd	1.11	4	nd	63	13.19

[a]Average of duplicate determinations

Table 3—**Analysis of variance** *to determine the effect of added nitrite on NTHZ formation*

Source	Sum of squares	Degrees of freedom	Mean square	F ratio
Total	1,232.86	47	—	—
Belly	193.40	7	27.68	103.96[a]
Treatment	789.48	2	394.74	22.69[a]
Belly × treatment	243.61	14	17.40	65.47[a]
Error	6.378	24	0.266	—

[a]Significant at $P < 0.01$

Table 4—**Effect of potential inhibitors** *on NTHZ formation in bacon*

	Concentration									
	Belly 1		2		3		4		5	
Inhibition	$NaNO_2$ (ppm)	NTHZ (ppb)	$NaNO_2$ (ppm)	NTHZ (ppb)	$NaNO_2$ (ppm)	NTHZ (ppb)	$NaNO_2$ (ppm)	NTHZ (ppb)	$NaNO_2$ (ppm)	NTHZ (ppb)
Control[a]	91	4.6	79	5.8	75	13.1	58	5.5	74	9.1
NaAsc	69	2.9	71	3.9	39	3.6	25	2.5	31	1.7
α-tocopherol	97	5.6	72	5.1	76	9.4	85	5.6	81	7.5
NaAsc/α-tocopherol	44	2.2	69	1.8	41	3.5	43	1.9	31	3.1
Liquid smoke (spray)	46	2.5	44	1.9	20	2.2	34	3.3	21	3.0
α-tocopherol (spray)	88	6.9	93	4.5	65	9.6	82	6.8	101	10.7

[a]Control contains 120 ppm $NaNO_2$ only

Table 5—**Analysis of variance** *to determine the effect of inhibitors on NTHZ formation in bacon*

Source	Sum of squares	Degrees of freedom	Mean square	F ratio
Total	521.26	59	—	—
Belly	85.30	4	21.33	177.04[a]
Treatment	330.46	5	66.09	12.97[a]
Belly × treatment	101.89	20	5.09	42.30[a]
Error	3.614	30	0.120	—

[a]Significant at $P < 0.01$

1979), and other phenols have either an inhibiting or catalytic effect on the nitrosation of several amines, depending upon their structure and the experimental conditions employed (Challis and Bartlett, 1975; Kurechi et al., 1980).

Since liquid smoke sprayed on the surface of bellies significantly inhibited NTHZ formation, we conducted two experiments to determine if liquid smoke added to the cure or sodium ascorbate sprayed on the belly surface also inhibited NTHZ formation. Statistical analysis of the data from these two experiments indicated that neither treatment significantly decreased NTHZ formation. The reason that the ascorbate spray treatment is ineffective in inhibiting NTHZ formation at the belly surface may be its rapid destruction by air oxidation, leaving little or no NTHZ available for nitrite destruction or for directly inhibiting the nitrosation reaction. The ineffectiveness of liquid smoke in the cure is probably

due to the sparse distribution of the inhibitory compounds throughout the entire belly, rather than being concentrated at the surface where NTHZ formation occurs. These results support our other data which indicate that a smoke component(s) reacts with a bacon precursor(s) to form NTHZ.

Can Reduce NTHZ Formation

In conclusion, our results show that NTHZ formation in bacon can be significantly reduced when using the currently permitted amount of sodium ascorbate (550 ppm) or when the belly is sprayed with liquid smoke solutions prior to and during processing. Since our results also indicate that cysteamine is the most likely precursor of NTHZ in bacon, the formation of a mutagenic product via the cysteamine–formaldehyde–nitrite pathway is highly probable. Further research is underway to determine if this mutagen is indeed a nitrosamine and if it can be inhibited in the same way as NTHZ.

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